FUNGI ISOLATION FROM LEAVES OF SOME MEDITERRANEAN EVERGREEN SCLEROPHYLLOUS SHRUBS. ENZYMATIC ACTIVITY OF THE ISOLATED FUNGI

by S. MARAKIS and S. DIAMANTOGLOU

University of Athens, Institute of General Botany,
157 84 Athens, Greece.

ABSTRACT - The young leaves of *Olea europaea* L. var. *silvestris* Brot., *Ceratonia siliqua* L., *Quercus coccifera* L. and *Pistacia lentiscus* L. are richer in nitrogen and tannins but poorer in carbohydrate contents compared to those of mature leaves. Forty fungal species belonging to 27 genera were isolated. The species isolated from mature leaves were about 2-4 times greater in number than the ones isolated from young leaves. *Alternaria alternata, Aspergillus carbonarius* and *Penicillium glabrum* were the most common fungi isolated during the survey. The greater number of fungal species was isolated from *O. europaea* leaves, while the smaller from *Q. coccifera*. Many fungi were common saprophytes while others were plant pathogens. The leaf tannin fraction precipitated by Tween 80 favored the growth of the fungi isolated from leaves rich in this tannin fraction. The tannase activity depended on fungal species and the leaves where fungi were isolated. The fungi isolated from *Q. coccifera* leaves presented the higher tannase activity. *Trichoderma viride* and *A. carbonarius* presented the higher cellulase and tannase activity respectively.

RÉSUMÉ - Les jeunes feuilles d' *Olea europaea* L. var. *silvestris* Brot., *Ceratonia siliqua* L., *Quercus coccifera* L. et *Pistacia lentiscus* L. sont plus riches en azote et en tanins mais plus pauvres en glucides que les feuilles âgées. Quarante espèces fongiques appartenant à 27 genres ont été trouvées. Les espèces fongiques isolées des feuilles âgées sont 2-4 fois plus nombreuses que celles des jeunes feuilles. *Alternaria alternata, Aspergillus carbonarius* et *Penicillium glabrum* sont les champignons les plus communs. Le plus grand nombre d'espèces fongiques est isolé des feuilles d' *O. europaea*, tandis que le plus petit nombre correspond à *Q. coccifera*. Plusieurs champignons sont des saprophytes communs tandis que d'autres sont des pathogènes des plantes. La fraction des tanins des feuilles, précipitée par le Tween 80, favorise la croissance des champignons isolés des feuilles riches en cette même fraction. L'activité tannase dépend des espèces fongiques et des feuilles d'où proviennent les champignons. Les champignons des feuilles de *Q. coccifera* présentent la plus haute activité en tannase. *Trichoderma viride* et *A. carbonarius* présentent respectivement la plus haute activité en cellulase et en tannase.

KEY WORDS : Maquis fungal flora, tannins, tannase, cellulase.

INTRODUCTION

Aiming the microbial degradation of tannins and lignocellulose, we undertook a fungal screening from leaves of some evergreen sclerophyllous shrubs (*Ceratonia siliqua* L., *Quercus coccifera* L., *Pistacia lentiscus* L., *Olea europaea* L. var. *silvestris* Brot.). These "maquis" were preferred for the significant tannin content of the first three species. Consequently, fungi with high tannin-lignocellulolytic activity could be isolated. Fungi with such abilities could be used for upgrading carob bean value. Deseeded carob pod contains: water soluble sugars (60%), lignin 12.5%, tannins 6%, cellulose 6% and hemicelluloses 4.8% (Marakis, 1980; Marakis et al., 1987).

The carob bean fungal flora was determined by Charpentié & Marakis (1980). Some of these fungi presented high tanninolytic activity (Marakis, 1980, 1985).

Saccardo (1898) carried out the first fungal survey on *C. siliqua*. He cited several fungi e. g. *Phyllosticta ceratoniae* Berk., *Septoria carrubi* Pass. and *Sphaerella ceratoniae* Pass. Sixty years later, several phytopathologists isolated some pathogenic fungi as: *Oidium ceratoniae* Comes. (Graniti, 1958), *Glomerella cingulata* (Stonem.) Spauld. & v. Schrenk (Martelli, 1961) and *Diplodina ceratoniae* Sarejanni (Demetriades et al., 1959). Recently, fungal species, belonging to 12 genera, were isolated from carob leaves and fruits for the first time in Portugal (Moreira, 1987). The fungus-host index for Greece (Pantidou, 1973) includes fungal species isolated from *O. europaea*, *C. siliqua*, *P. lentiscus* and *Q. coccifera*.

The above mentioned studies (besides that of Charpentié & Marakis, 1980) have to be mainly with parasitic and pathogenic fungi. Yet, the distribution of fungi occurring on leaves of the under study evergreen sclerophyllous shrubs in Greece, as well as the tannino-cellulolytic activity of these fungi have not been studied.

Aim of this study is to determine fungi occurring on the young, mature and fallen leaves (litter) of *O. europaea* var. *silvestris*, *C. siliqua*, *P. lentiscus* and *Q. coccifera* shrubs, as well as the tannase and cellulase activity of the isolated fungi.

MATERIALS AND METHODS

Preparation of leaf extract and TPT fraction

The tannin fraction precipitated by Tween 80 (TPT fraction) was prepared as follows: 0.4kg of freeze-dried and milled (2mm sieve) leaves of each examined shrubs was mixed with 2.5l of deionized water and autoclaved for 30 min at 121°C. The slurry was passed through cheesecloth and the extracted leaves resuspended in 1.5l of deionized water and autoclaved once again. 0.15g sodium pyrosulfite has been added to prevent tannin oxidation, and the two filtrates were combined (leaf extract).

The TPT fraction was precipitated by addition of 5ml Tween 80 per litre of leaf extract. This tannin fraction was filtrated and dried by lyophilization.

Media

1. For isolation of fungi, many common standard or selective media [Czapek-Dox, potato dextrose, malt extract, lupin stems, corn meal, oatmeal, hay-infusion agar, etc. (Miller et al., 1957; Raper & Fennell, 1965; Booth, 1971; von Arx, 1981; Burns & Slater, 1982)] were used. The media A1-4 (of Czapek-Dox type) also used, contained 10% w/v leaf powder rather than 3% sucrose. So, the indexes 1,2,3,4 represent the leaf powder of *O. europaea*, *C. siliqua*, *Q. coccifera* and *P. lentiscus* respectively. Chloramphenicol (0.05 mg/ml of medium) was added in order to supress bacterial growth.

2. Medium B, contained (g/l): TPT fraction, 20; $(NH_4)_2$ SO_4, 5; K_2 HPO_4, 1; $MgSO_4.7H_2O$, 0.5: KCl, 0.5: $ZnSO_4$, 0.01; $CuSO_4.5H_2O$, 0.005; biotin, 0.04; thiamine, 1; pyridoxine hydrochloride, 0.5; and nicotinic acid, 0.5. The pH was adjusted to 5-5.5.

3. Medium C: the salts of medium were the same with those used by Peitersen (1975), supplemented with 2% cellulose.

4. Medium D: its composition was the same with the medium used by Yamada et al. (1968).

The media were sterilized by autoclaving (15 min., 121°C).

Isolation of fungi

During the spring of 1985, 150 leaves [50 of each: fallen (usually drying or decaying), mature and young] were sampled from various parts of each shrub, and brought to the laboratory in sterilized glass bottles. Then, the leaves were aseptically cut into pieces 0.5-1.5cm long. Samplings were repeated the next two years from the same shrubs and season, from several districts of Greece.

Five pieces from the leaves of each category were mixed with 10ml of medium (43°C) into a petri dish by simultaneous stirring. Another five leaf pieces were placed on solid medium. The petri dishes were grouped according to the used growth medium. Duplicates of each petri dish group were incubated at ten different temperatures (between 22.5-45°C). The growing colonies were purified by several methods (direct isolation, dilution-plate etc.) as they are described by Raper & Fennell (1965), Booth (1971), Waterhouse (1971) and Charpentié & Marakis (1980).

The pure fungal isolates were preserved by lyophilization (Marakis, 1980). These fungi were identified according to the classification tables by Raper & Thom (1949), Barnett (1955), Raper & Fennell (1965), Ainsworth et al. (1973), von Arx (1981). In some cases more specialized monographs or descriptions of fungal species were used. No attempt was made to determine relative frequency of occurrence of any fungal species in a leaf sample.

Batch cultivation

1. Fungal growth in medium B: the fungi were grown in 300ml Erlenmeyer flasks containing 50ml of medium B. These flasks were inoculated with 10^6 spores/ml of medium and incubated on reciprocal shaker (120 strokes per min) for 96 h at optimum temperature for each fungal species. The biomass was harvested by method of Marakis (1985). Each of the above experiments was run in triplicate (3 flasks per run). The results were presented as mean values $\pm$ standard error.

2. Enzyme production: the culture conditions for cellulase production in medium C were the same as those above mentioned with an incubation time extension to 5 days. For tannase production, the fungi were cultured in medium D under conditions which have been described by Yamada et al. (1968). The enzyme tests were made in triplicate at least on two different occasions.

All isolations belonging to the same fungal species were examined in order to reveal any strain relative to biomass production and enzyme activities.

Analytical methods

- Water soluble total sugars in leaf extracts were determined by the method of Dubois et al (1956) after tannin removal according to the *Association of Official Agricultural Chemists* (AOAC, 1970) method.

- Total nitrogen (TN) was estimated by the method of Varley (1966).

- Protein nitrogen (PN) was calculated by comparing non protein nitrogen (NPN), to TN. For the calculation of NPN, hexosamines and nucleic acids were assumed to contain 7.8 and 15% N, respectively (Smith et al., 1975).

- Hexosamines were determined by the method of Rondle & Morgan (1955).

- Nucleic acids were extracted by the method of Delaney et al. (1975). RNA was estimated by the method of Gottlieb & Van Etten (1964) and DNA by the diphenylamine method (Dische, 1955) using baker's yeast RNA and calf thymus DNA as standards.

- Tannins were determined according to Marakis (1985).

- Cellulose was estimated by the method of Updegraff (1969).

Enzyme assays

- Filter paper (F.P.) cellulase activity was determined by the Peitersen (1975) method. One filter paper unit ($U.ml^{-1}$) corresponds to 1 mg/ml/h glucose.

- Tannase activity was determined by Yamada et al. (1968) method.

RESULTS AND DISCUSSION

Composition of leaves

The results (composition of young and mature leaves presented in Table I reveal that:

Shrubs	*O. europaea*		*C. siliqua*		*P. lentiscus*		*Q. coccifera*	
	young	mature	young	mature	young	mature	young	mature
Total nitrogen	2.6	2.0	2.5	1.8	2.8	0.9	2.5	0.8
Protein nitrogen	1.9	1.5	1.7	1.0	1.9	0.3	1.8	0.2
Water soluble sugars	2.1	3.6	1.8	3.8	1.6	3.5	1.7	3.5
Cellulose	18.9	32.4	17.8	30.1	15.3	28.4	17.3	39.2
Total tannins	5.7	3.0	16.2	8.1	17.1	8.8	19.3	11.1
TPT fraction	0.2	0.01	4.8	1.0	7.3	1.2	8.6	2.1

Table I. - Composition (% on dry weight) in nitrogen, water soluble sugars, cellulose and tannins of young and mature leaves of *O. europaea* var. *silvestris*, *C. siliqua*, *P. lentiscus* and *Q. coccifera*.

Tableau I. - Composition (% sur poids sec) en azote, sucres solubles dans l'eau , cellulose et tanins des feuilles jeunes et âgées d' *O. europaea* var. *silvestris*, *C. siliqua*, *P. lentiscus* et *Q. coccifera*.

1) The nitrogen percentage generally decreases with the development of the leaves. This is in agreement with the data reported by Diamantoglou & Kull (1988). The nitrogen content is similar between the young leaves of all four shrubs examined, while the total nitrogen content of the mature leaves in *O. europaea* and *C. siliqua* was about twofold of that in *P. lentiscus* and *Q. coccifera*.

2) Significant variations in the water soluble sugar content either between young leaves or the mature ones were not observed. The soluble sugar percentage of the mature leaves was about twofold of that of the young leaves. Diamantoglou & Meletiou-Christou (1980) indicated that the soluble sugars in mature leaves were higher than those of young leaves in *Pistacia vera* and *P. terebinthus*.

3) The cellulose content was found higher than 69-127% in mature leaves compared to that of young leaves. This difference was as expected. The higher cellulose percentage (39.2%) was observed in mature leaves of *Q. coccifera*, while the lower one (28.4%) was in *P. lentiscus* mature leaves. This is in agreement with the raw fibre contents reported by Diamantoglou & Kull (1988) for the mature leaves of the above shrubs.

4) The leaves of *Q. coccifera*, *P. lentiscus* and *C. siliqua* contain about threefold total tannins compared to those of *O. europaea* leaves. The percentage of total tannins was 74-100% higher in the young leaves than in the mature ones. But, if the tannin content is calculated on fresh weight of leaves, the above difference of the tannin percentage between young and mature leaves is significantly lower (20-50%). The TPT fraction, which possibly consists of condensed tannin precursors, was ranged between 3.5-45% (on

total tannins) in young leaves and 0.3-19% in mature leaves. This tannin fraction is significantly lower in *O. europaea* leaves compared to that of the other examined shrubs. This is in accordance with the results reported by Christodoulakis (1984) with the exception of *O. europaea* leaves where the histochemical reactions he employed were negative. The higher concentrations of tannins in the young leaves are possibly an additional protection for these leaves at a stage of their life when they have not yet been hardened. This protection is effective not only against drought but also against herbivores. However, the presence of tannins in the leaf epidermis of most of the evergreen sclerophyllous species (Mitrakos & Christodoulakis, 1981) indicates the role of tannins which seems to be equally significant for the mature leaves. The development of other protective mechanisms (hairs, spiny margins, etc.) possibly decrease the importance of the tannins in the mature leaves.

Fungal species

Table II presents the fungi isolated from young, mature and fallen leaves of some evergreen sclerophyllous shrubs. Some of these (*Colletotrichum gloeosporioides, Phomopsis oblonga, Epicoccum nigrum, Trichoderma roseum, Alternaria alternata*, etc.) were isolated from the examined shrubs for the first time in Greece. Many species occurred on more than one shrub species, while others were isolated from only a single species. The fungal species isolated from the young leaves of a certain shrub, most of the time occurred on the mature and fallen leaves of the same shrub species. The fungal species, we isolated from mature leaves were about 2-4 times greater in number than the ones isolated from young leaves. This is due perhaps to the short period of time of the existence of young leaves or to their higher TPT fraction content, which may affect the growth of the isolated fungi in different ways related to the concentration of this particular tannin fraction in the leaves (see Tab. III). Many of fungi isolated during this study have been previously reported from the examined shrub species (Maire & Politis, 1940; Pantidou, 1973; Charpentié & Marakis, 1980; Moreira, 1987).

Alternaria alternata was isolated from all categories of examined leaves. It would be expected, because this microorganism is a cosmopolitan saprophytic colonist of plant surfaces (decaying leaves, fruits, etc.). The species *Aspergillus carbonarius* and *Penicillium glabrum* occurring on the leaves of tannin rich shrubs, have been isolated from carob beans (Charpentié & Marakis, 1980). *Trichoderma harzianum* often occurred in warm districts, while *T. viride* was isolated from cool regions. On leaves, where *Trichoderma* species were isolated, less isolates of other species were recorded. This is possibly due to *Trichoderma*, which, as fast-growing microorganisms, use the nonstructural carbohydrates in a fast way; or fungi may secrete inhibitors, antagonistic for other fungal species. *C. gloeosporioides* was isolated from dark brown lesions on the carob leaves. This fungus would be considered as a part of the surface fungal flora (mainly leaf-inhabiting fungus) of *C. siliqua* because it is presented on the premature, mature and fallen leaves. *P. oblonga* possibly sporulates as a saprobe in dead host materials and then infects young and mature leaves. Most infections of *Taphrina caerulescens* occurred

Shrubs	*C. europaea*	*C. siliqua*	*Q. coccifera*	*P. lentiscus*
Young leaves	*Alternaria alternata* (Fr.) Keis. *Aspergillus niger* Van Tiegh. *Fusarium moniliforme* Sheld. *Mucor racemosus* Fres. *Penicillium notatum* West. *Phyllosticta oleae* Pet. *Rhizopus stolonifer* (Eh. ex Fr.) V. *Trichothecium roseum* Link	*A. alternata* *Aspergillus carbonarius* Bain. *Mucor genevensis** Lendner *Penicillium glabrum* (Weh.) West. *Phomopsis oblonga* Desm.	*A. alternana* *A. carbonarius* *P. glabrum* *Taphrina caerulescens** Tul.	*A. alternata* *A. carbonarius* *P. glabrum*
Mature leaves (1)	*Aspergillus flavus* Link *Aspergillus fumigatus** Fr. *Capnodium oleae** Arn. *Cladosporium herbarum* Link ex Fr. *Cycloconium oleaginum* Cast. *Penicillium cyclopium* West. *Phoma herbarum* Westend *Trichoderma viride* Per. ex Fr. *Phytophthora megasperma* Drech.	*A. niger* *Cladosporium cladosporioides* (Fr.) de Vr. *Colletotrichum gloeosporioides* Penz. *Diplodia ceratoniae* Politis *Epicoccum nigrum* Link *R. stolonifer* *Septoria* sp.* *Trichoderma harzianum* Rif. ag. *T. roseum*	*A. niger* *C. cladosporioides* *Coccomyces tentatus* Kun. & Sch. *Coniothyrium quercinum* Politis *Acremonium strictum* W. Gams *T. harzianum*	*A. niger* *Capnodium lentisci* Pass. *C. cladosporioides* *M. genevensis* *Phoma lentisci* Pass. *P. oblonga* *R. stolonifer* *Septoria pistaciae** Desm. *T. harzianum*
Fallen leaves (2)	*Coleophoma* sp. *Penicillium funiculosum* Thom	*Aspergillus terreus* Thom *Mucor mucedo* Fr.	*R. stolonifer* *T. viride*	*Aposphaeria lentisci* (Dur. & Mont.) Pat. *Paecilomyces variotii* Bain.

Table II - Fungi isolated from young, mature and fallen leaves of *O. europaea* var. *silvestris*, *C. siliqua*, *Q. coccifera* and *P. lentiscus*.

Tableau II - Champignons isolés des feuilles jeunes, âgées et tombées d'*O. europaea* var. *silvestris*, *C. siliqua*, *Q. coccifera* et *P. lentiscus*.

(1) In addition to the fungi mentioned above, excluding those with asterisk (*), the following species were also isolated.

(2) In addition to the fungi isolated from mature leaves, excluding those with asterisk (*), the following species were also isolated.

Examined parameters / Fungi	Biomass dry weight (mg/flask)	Tannase total units ($x10^3$)	F.P. cellulase ($U.ml^{-1}$)
Acremonium strictum	16.0±0.9	10.8	0.8
Alternaria alternata (O.e.)	5.3±0.5	0.9	NP
Alternaria alternata (C.s.)	75.0±1.2	25.6	0.4
Alternaria alternata	105.1±2.2	40.2	1.7
Aposphaeria lentisci	NG	NP	0.9
Aspergillus carbonarius	136.1±2.6	175.3	1.1
Aspergillus flavus	45.0±1.6	35.4	NP
Aspergillus fumigatus	NG	NP	0.4
Aspergillus niger (O.e.)	5.1±0.8	0.6	NP
Aspergillus niger	37.1± 2.1	16.1	0.7
Aspergillus terreus	5.5±1.0	0.8	1.8
Capnodium lentisci	NG	NP	NP
Capnodium oleae	NG	NP	NP
Coccomyces tentatus	17.2±1.6	12.1	2.1
Cladosporium herbarum	NG	0.2	0.7
Cladosporium cladosporioides	15.3±0.8	7.9	NP
Colletotrichum gloeosporioides	27.5±1.4	14.2	NP
Coleophoma sp.	NG	NP	0.8
Coniothyrium quercinum	25.6±2.1	13.3	2.2
Cycloconium oleaginum	2.8±0.6	0.3	NP
Diplodia ceratoniae	9.4±1.1	5.1	NP
Epicoccum nigrum	7.2± 0.6	2.1	0.4
Fusarium moniliforme	NG	NP	1.9
Mucor genevensis	75.4±1.9	41.2	NP
Mucor mucedo	18.1±1.2	9.3	2.0
Mucor racemosus	6.2±0.8	0.4	NP
Paecilomyces variotii	20.6±1.7	10.1	1.8
Penicillium cyclopium	4.5±0.9	0.7	NP
Penicillium frequentans	98.6±1.8	135.1	2.2
Penicillium funiculosum	NG	0.3	1.3
Penicillium notatum	5.8±1.1	0.9	NP
Phoma herbarum	3.0±0.7	0.4	0.6
Phoma lentisci	7.9±1.0	4.5	NP
Phomopsis oblonga	80.0±1.5	65.7	2.1
Phyllosticta oleae	3.2±0.7	0.2	NP
Phytophthora megasperma	NG	NP	1.2
Rhizopus nigricans	9.2±1.3	4.3	0.9
Septoria sp.	3.6±0.8	0.3	NP
Septoria pistaciae	6.2±1.0	1.7	NP
Taphrina caerulescens	76.1±1.2	45.3	2.3
Trichoderma harzianum	32.2+1.4	16.1	3.2
Trichoderma viride	NG	NP	3.7
Trichoderma roseum	NG	NP	NP

O.e., C.s. = fungi isolated from *O. europaea* and *C. siliqua* leaves respectively, NG = no growth, NP = microorganisms did not present enzyme activity.

Table III - Biomass dry weight of the isolated fungi cultured in medium B for 96h, and their enzyme activity (tannase and F.P. cellulase).

Tableau III - Poids sec de biomasse des champignons isolés cultivés en milieu B pendant 96h, et activités de leurs enzymes (tannase et P.F. cellulase).

on leaves after emergence from buds. Thus, the presence of this microorganism on young leaves may be justified due to the fact that undifferentiated tissues are more susceptible.

Fungal growth and enzyme activities

Table III shows the fungal growth in medium B and the tannase and filter paper (F.P.) cellulase activities of the isolated fungi. The species, isolated from young leaves of tannin rich shrubs (*C. siliqua*, *Q. coccifera*, *P. lentiscus*), presented the greater biomass production (75-136 mg/flasks), while those isolated from *O. europaea* leaves or from some leaf samples of other examined shrubs failed to grow or usually presented a much lower growth (> 10mg/flask). However, the fungal species: *Cladosporium cladosporioides*, *Acremonium strictum*, *Coccomyces tentatus*, *Mucor mucedo*, *Paecilomyces variotii*, *Coniothyrium quercinum*, *Colletotrichum gloeosporioides*, *Trichoderma harzianum*, *Aspergillus niger* and *A. flavus* isolated from mature and fallen leaves presented a noticeable biomass ranging between 15-45 mg/flask. Several plant pathogen species, mainly those isolated from *Q. coccifera*, presented higher than 15mg of dry biomass per flask. The fungi grown in medium B might possess an enzyme system for degradation and utilization of the TPT fraction. This tannin fraction did not favor the growth of isolated fungi from leaves with poor TPT content. This is possibly due to the different adaptation of the isolated fungi from leaves containing various concentrations of TPT fraction. An increase of incubation time for all cultures did not change the results. The chemical analysis of the TPT fraction may possibly elucidate the way in which this tannin fraction is assimilated by the isolated fungi.

The isolations from different leaf categories belonging to the same fungal species presented similar growth and enzyme activity except the isolates of *Alternaria alternata* and *Aspergillus niger*. This study revealed three strains of *A. alternata* and two of *A. niger*. The strains of these fungal species isolated from *O. europaea* leaves presented much lower values of the examined parameters compared to those of the strains isolated from leaves of tannin rich shrubs.

The tannase activity depended on the fungi species and the leaves where fungi were isolated from. The differences of tannase activity between fungal species presented similar variations to those of the biomass growth. So, fungal species produced high biomass growth showed high tannase activity. *A. carbonarius* and *P. glabrum* presented the higher tannase values, 175.3 and 135.1 total units (x 10^3), respectively. The plant pathogen species *P. oblonga* and *T. caerulescens* presented high tannase activity as well. It is noticeable that fungi isolated from *Q. coccifera* leaves presented higher tannase activity. This is possibly due to the fact that *Q. coccifera* leaves are richer in tannins than those of other examined shrubs. Tannase activities higher than 20 total units(x 10^3) of some microfungi is in accordance with the tanninolytic ability of fungi belonging to the same species isolated from carob bean (Marakis, 1980). The tannase activity [45 total units (x 10^3)] of *A. flavus* is lower than that reported by Yamada et al. (1968) for a strain of this fungal species.

Twenty four fungal species, 60% of those isolated, presented cellulase activity. All fungi isolated from fallen drying and decaying leaves showed considerable cellulolytic activity. *Trichoderma* presented the higher values (3.2-3.7 $U.ml^{-1}$) of F.P. cellulase. This should be expected, because these fungal species are considered to be among the cellulolytic microorganisms. In preliminary experiments these fungi presented a low ligninolytic activity. Thereafter, it is someway enigmatic that in spite of the considerable cellulolytic activity of *Trichoderma* species their ability to degrade lignin is weak. These species although can utilize a diverse range of substrates, *T. viride* cannot grow in medium B. The saprophytes generally presented a higher cellulase activity, but some plant pathogens (*C. tentatus*, *C. quercinum*, *P. oblonga* and *T. caerulescens*) showed a noticeable cellulolytic activity (>2 $U.ml^{-1}$) as well. These pathogens fungi presented a high tannase activity. A research on the relationship between tannin-lignocellulolytic ability and pathogenicity of these fungal species is useful to be undertaken.

CONCLUSION

The isolated fungal species generally presented an adaptation to their environment. So, fungi isolated from leaves of tannin rich shrubs showed higher tannase activity than that of the fungal species isolated from other leaf categories examined. The smaller number of fungal species occurred on the tannin rich leaves. This is perhaps due to the inhibitory effects that tannins have on microbial growth.

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